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MINIREVIEW ARTICLE

Inflammation, carcinogenesis and neurodegeneration studies in transgenic animal models for polyamine research

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Abstract Natural polyamines (PA) are cationic molecules affecting cell growth and proliferation. An association between increased polyamine biosynthesis and inflammation-induced carcinogenesis has been recognised. On the other hand, there are indications that inflammatory stimuli can up-regulate polyamine catabolism and that altered polyamine metabolism could affect pro- and anti-inflammatory cytokines. Since the polyamine content is strictly related to cell growth, a consistent number of evidences relate polyamine metabolism dysfunction with cancer. The increase of polyamine levels in malignant and proliferating cells attracted the interest of scientists during last decades, addressing polyamine depletion as a new strategy to inhibit carcinogenesis. Several studies suggest that PA also play an important role in neurodegeneration, but the mechanisms by which they participate in neuronal death are still unclear. Furthermore, the role of endogenous PA in normal brain functioning is yet to be elucidated. The consequences of an alteration of polyamine metabolism have also been approached in vivo with the use of transgenic animals overexpressing or devoid of some enzymes involved in polyamine metabolism. In the present work we review the experimental investigation carried out on inflammation, cancerogenesis and neurodegeneration using transgenic animals engineered as models for polyamine research.

Keywords Polyamine metabolism · Transgenic animals · Inflammation · Carcinogenesis · Neurodegeneration

Abbreviations

AcSpd	N^1-acetylspermidine
AcSpm	N^1-acetylspermine
AKI	Acute kidney injury
AP	3-Aminopropanal
APAO	N^1-acetylpolyamine oxidase
AZ	Antizyme
DFMO	α-Difluoromethylornithine
DMBA	7,12-Dimethylbenz(a)anthracene
FAD	Flavin-adenine-dinucleotide
GFP	Green fluorescent protein
Glu	Glutamate
GluR	Glutamate receptor
IL-10	Interleukin-10
LPS	Bacterial lipopolysaccharide
ML	Maximum likelihood
MDL72527	N^1, N^4-Bis(2,3-butadienyl)-1, 4-butanediamine
NMDA	*N*-methyl-D-aspartate
ODC	Ornithine decarboxylase
ODCER	Estrogen receptor ligand-binding domain
PA	Polyamines
PAO	Polyamine oxidases
PTZ	Pentylenetetrazol
Put	Putrescine

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ROS	Reactive oxygen species
TPA	12-*O*-tetradecanoylphorbol-13-acetate
Spd	Spermidine
SMO	Spermine oxidase
SMS	Spermine synthase
SPDS	Spermidine synthase
Spm	Spermine
SSAT	Spermidine/spermine N^1-acetyltransferase
4OHT	4-Hydroxytamoxifen

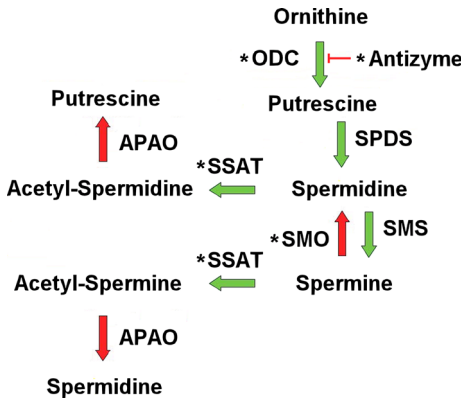

Fig. 1 Metabolic pathway of eukaryotic polyamines.The biosynthetic reactions are shown in *green arrows* and the catabolic reactions are shown in *red arrows*. *APAO* acetylpolyamine oxidase, *ODC* ornithine decarboxylase, *SSAT* spermidine/spermine *N1*-acetyltransferase; *SMO* spermine oxidase, *SMS* spermine synthase, *SPDS* spermidine synthase. *Asterisks* indicate enzymes which activity has been changed by overexpression or knockout of the corresponding genes in transgenic animals (color figure online)

Introduction

The polyamines (PA) putrescine (Put), spermidine (Spd), and spermine (Spm) are natural aliphatic polycations ubiquitous in living organisms and essential for cell growth, proliferation, and differentiation. Polyamine homoeostasis in mammalian cells are finely regulated by a complex system of regulatory mechanisms affecting synthesis and degradation, as well as membrane transport (Polticelli et al. 2012). Polyamine biosynthesis is step-limited by the action of two enzymes; the ornithine decarboxylase enzyme (ODC) which produces Put by decarboxylation of ornithine, and the *S*-adenosylmethionine decarboxylase (SAMDC) responsible of the production of the aminopropyl moiety by *S*-adenosylmethionine decarboxylation. The two specific aminopropyl transferases, spermidine synthase (SPDS) and spermine synthase (SMS), synthesise Spd and Spm by adding the aminopropyl group respectively to Put and to Spd (Amendola et al. 2009 and references therein). The PA catabolism is dependent on the activity of the enzyme spermidine/spermine N^1-acetyltransferase (SSAT), able to transfer an acetyl group from acetyl-coenzyme A to the N^1 position of both Spd and Spm. The N^1-acetylspermidine (AcSpd) and the N^1-acetylspermine (AcSpm) are then oxidised by the FAD-dependent enzyme N^1-acetylpolyamine oxidase (APAO) to respectively produce Spd and Spm, 3-aceto-aminopropanal and hydrogen peroxide (H_2O_2) (Tavladoraki et al. 2011). Another catabolic enzyme is the spermine oxidase (SMO, EC number 1.5.3.3), which specifically oxidises Spm producing Spd, the aldehyde 3-aminopropanal (AP) that spontaneously turns into acrolein, and H_2O_2 (Fig. 1) (Cervelli et al. 2003, 2012, 2013a). Cloning of genes involved in PA metabolism has allowed the generation of genetically modified mice and rats overproducing or lacking proteins encoded by these genes. The consequences of gene overexpression can be studied either ubiquitously or in a tissue-specific manner in transgenic animals. Transgenic technology has allowed us to better understand how inflammation, tumorigenesis and neurodegeneration link to polyamine metabolism, which will be discussed in the corresponding sections of this review and summarised in Table 1.

Since PA homeostasis appears to be greatly altered during inflammation, transgenic animals with changed PA metabolism have been employed to study the relationship between inflammation processes and PA. On this issue, a number of experiments involving transgenic rodents overexpressing or lacking the *SSAT* gene were reported in the last decade (Hyvönen et al. 2007; Zahedi et al. 2010; Pirnes-Karhu et al. 2012). For instance, SSAT enzyme overexpression in rats has been proved to decrease the amount of Spd and Spm and to generate acute pancreatitis (Hyvönen et al. 2007). In accord with the pro-inflammatory role of SSAT enzyme, experiments conducted in SSAT-deficient mice, subjected to endotoxin-induced acute kidney injury (AKI) following intraperitoneal injection of bacterial lipopolysaccharide (LPS), appeared to be less prone to tissue damage (Zahedi et al. 2010). Controversially, an anti-inflammatory effect of SSAT was obtained using transgenic mice overexpressing SSAT treated with LPS, which showed a high level of cytokine IL-10 (Pirnes-Karhu et al. 2012), testifying the complexity of the inflammatory response and PA metabolism.

Altered PA metabolism is also associated with normal and neoplastic growth (Jänne et al. 2005). Thus, a transgenic mouse model overexpressing ODC resulted to be more sensitive to carcinogens than wild-type mice in developing skin tumours (Chen et al. 2000). On the other hand, inducible ODC transgenic mice after topical drug administration also showed the onset of tumorigenesis (Hayes Hayes et al. 2011). In agreement with these results, mice that express a constitutively active antizyme (AZ)-1,

Table 1 Transgenic animal models for polyamine metabolism and major findings in connection with inflammation, carcinogenesis and neurodegeneration

Target gene	Promoter/expression	Major phenotypic changes	References
Inflammation			
SSAT	Metallothionein I promoter/inducibile expression	Acute pancreatitis development	Alhonen et al. (2000); Rasanen et al. (2002); Hyvönen et al. (2007)
SSAT	SSAT promoter/ constitutive expression	More protection to LPS induced acute kidney injury	Pietilä et al. (1997); Pirnes-Karhu et al. (2012)
SSAT	Knock out	More protection to LPS induced acute kidney injury	Tucker et al. (2005); Zahedi et al. (2010)
Carcinogenesis			
ODC (truncated form)	K6 promoter/constitutive expression	Promotion of skin tumorigenesis after DMBA treatment	Megosh et al. (1995); Chen et al. (2000)
ODC	Involucrin promoter/ inducibile expression	Activation of specific pathways that support and promote tumorigenesis	Lan et al. (2005); Hayes et al. (2011)
AZ-1	K6 promoter/constitutive expression	Reduced susceptibility to carcinogens	Feith et al. (2001, 2007)
AZ-1	K5 promoter/constitutive expression	Double transgenic mice K5-AZ1/p53$^{+/-}$ are less prone to develop oral cavity and oesophagus tumours than control	Feith et al. (2001, 2013)
SSAT	K6 promoter/constitutive expression	Promotion of skin tumorigenesis after DMBA and TPA treatment	Coleman et al. (2002); Wang et al. (2007)
SSAT and AZ-1	K6 promoter/constitutive expression	Reduction of carcinogenesis after DMBA and TPA treatment	Wang et al. (2007)
SSAT	SSAT promoter/ constitutive expression	Promotion of intestinal and colon tumorigenesis in double transgenic *Apc*$^{Min/+}$*Sat1*^{tg}	Moser et al. (1990); Pietilä et al. (1997); Tucker et al. (2005); Berger et al. (2007)
SSAT	Knock-out	Reduction of intestinal and colon tumorigenesis in double transgenic mice Sat1$^{-/-}$/Apc$^{Min/+}$	Moser et al. (1990); Tucker et al. (2005); Berger et al. (2007)
SSAT	SSAT promoter/ constitutive expression	Reduction of prostate tumorigenesis in double transgenic mouse TRAMP/SSAT	Greenberg et al. (1995); Pietilä et al. (1997); Kee et al. (2004)
Neurodegeneration			
ODC	ODC promoter/ constitutive expression	Neuroprotection from physically or chemically induced seizure activity, neuroprotection from transient focal cerebral ischaemia, impaired spatial learning and memory	Alhonen et al. (2009); Halmekytö et al. (1991); Halonen et al. (1993); Lukkarinen et al. (1998)
SSAT	SSAT promoter/ constitutive expression	Neuroprotection from chemically induced seizure activity, impaired spatial learning, hypomotoric activity and less aggressiveness	Pietilä et al. (1997); Kaasinen et al. (2000, 2003, 2004); Alhonen et al. (2009)
SMO	CMV promoter/brain neocortex expression	Enhanced neurodegeneration during ageing and after kainic acid treatment, strong astroglial and microglial activation in neocortex during ageing	Cervelli et al. (2013a, b)

a protein that reduces ODC and PA transport, were more protected than the wild-type in response to carcinogens. Therefore AZ expression is able to reduce the susceptibility to tumour promoters (Feith et al. 2007, 2013). Regarding SSAT transgenic mice, its overexpression after carcinogens treatment can, in some cases, promote tumour development, while in others leads to an inhibition of tumorigenesis (Kee et al. 2004; Tucker et al. 2005; Berger et al. 2007). Consequently, the role of SSAT on carcinogenesis is context-dependent, and its overexpression can increase or decrease PA metabolic enzymes (Berger et al. 2007).

Several evidences suggest an important beneficial role for an increase of Put level in the brain of mice overexpressing ODC and SSAT genes. The use of transgenic animals turned out very useful to understand the role of Put accumulation in the brain, which is neuroprotective against ischaemia/reperfusion damage and elevates seizure threshold, although provoking impaired spatial learning. However, a different scenario is displayed by SMO overexpressing mice compared to ODC and SSAT overexpressing mice; in fact no sign of neuroprotection can be detected. On the contrary, an enhanced neurodegeneration during ageing and following kainate (KA) injection can be observed in *JoSMOrec* mice.

Inflammation

Since PAs are involved in inflammation processes, and viceversa, inflammatory stimuli could regulate PA catabolism, transgenic animals have been used as genetic tools to investigate this relationship (Pirnes-Karhu et al. 2012). Different works in the past reported an increase of the PA levels in inflamed tissues caused by infections and injuries (Zhang et al. 2000; Gobert et al. 2002), as well as an association between increased PA biosynthesis and inflammation-induced carcinogenesis (Amendola et al. 2009). Transgenic models overexpressing SSAT have been used to elucidate the link existing between PA depletion, after activation of PA catabolism, and pancreatitis (Alhonen et al. 2000; Hyvönen et al. 2007). SSAT overexpressing rats, under the control of a heavy metal inducible metallothionein promoter, showed a profoundly altered PA pool in pancreas when treated with nontoxic doses of zinc (Alhonen et al. 2000; Uimari et al. 2012). Accumulation of Put and decrease in Spd and Spm content, as well as increase in pancreatic SSAT activity and acute pancreatitis development were observed in a zinc dose-dependent manner (Alhonen et al. 2000; Uimari et al. 2012). A direct link between PA depletion and pancreatitis was further demonstrated by the report that in the same transgenic model a preceding administration of 1-methyl-Spd, a metabolically stable substitute for Spd, could prevent zinc-induced pancreatitis (Rasanen et al. 2002). A work carried out by Hyvönen et al. (2007) in pancreatic acinar cells has shown how the activity of cathepsin B, a tripsinogen activator of early events in the onset of pancreatitis, is linked to SSAT overexpressing and PA depletion.

Pre-treatment with 1,12-bismethyl-Spm, a stable analogue of Spm, partly metabolised in 1-methyl-Spd, reduced to a great extent the pancreatic activity of cathepsin B and completely blocked trypsinogen activation, thereby demonstrating a direct correlation between PA depletion and trypsinogen activation (Hyvönen et al. 2007). Moreover, Alhonen et al. (2000) demonstrated that H_2O_2 derived from acetylated PAs oxidation is not responsible for the inflammatory process, since inhibition of APAO by N^1,N^2-bis(2,3-butadienyl)-1,4-butanediamine (MDL72527), a specific inhibitor of SMO and APAO (Bellelli et al. 2004; Bianchi et al. 2006), did not alleviate pancreatitis. In a different genetic system, SSAT-deficient mice (Tucker et al. 2005) subjected to endotoxin-induced AKI, appeared to be more protected against the damage, following intraperitoneal injection of LPS (Zahedi et al. 2010). Transgenic $SSAT^{-/-}$ mice showed reduced level of blood creatinine and better preserved kidney functions in comparison with wild-type controls. Among PAs, only Put content appeared to be different between $SSAT^{-/-}$ and wild-type mice, being increased in control animals. Zahedi et al. (2010) suggested that this difference in Put content was due to SSAT induction in control mice in response to LPS stimulus, since ODC activity resulted to be not changed. The authors proposed that the increased kidney damage in wild-type animals was a consequence of oxidative PA catabolism mediated by the concerted action of SSAT and APAO, through the production of toxic products (such as H_2O_2, AP and acrolein). In supporting this explanation, mice subjected to endotoxin-induced AKI pre-treated with MDL72527 showed a decrease in the levels of creatinine in $SSAT^{-/-}$ mice. To summarise, ablation of SSAT and/or inhibition of APAO significantly reduced the ratio of oxidised glutathione to reduced glutathione (GSSG/GSH), otherwise observed after LPS exposure, supporting the protective role of SSAT in this genetic context (Zahedi et al. 2010). On the contrary, different results have been obtained using SSAT overexpressing mice (Pietilä et al. 1997) subjected to endotoxin-induced AKI, where the increase of SSAT has shown an anti-inflammatory acute response to LPS. In fact, serum levels of the main pro-inflammatory cytokines IL-1β and IFN-γ were significantly lower in SSAT overexpressing mice during the acute phase, while levels of the anti-inflammatory cytokine IL-10 increased after LPS injection. On the other hand, a clear protected phenotype was not detected in SSAT overexpressing transgenic animals, since the serum creatinine

level was higher and an increase of liver submicroscopic injury was observed (Pirnes-Karhu et al. 2012). All together these results indicate that alteration of the PA metabolism can determine complex and conflicting effect on the inflammatory response.

Carcinogenesis

Altered PA biosynthesis and catabolism are associated with normal and neoplastic growth (Jänne et al. 2005). Genetic and pharmacological studies that link PA content with tumourigenesis have been carried out using mice in which the PA metabolic pathway was modified by overexpression or deletion of PA regulatory enzymes and proteins (Pegg et al. 2003). Mice overexpressing ODC have mainly been used as animal models in cancer research experiments. In particular, the K6-ODC transgenic mouse model overexpressing a stable truncated form of ODC under the bovine keratin K6 promoter was generated by Megosh et al. (1995). The K6-ODC mice treated with 7,12-dimethylbenz(a)anthracene (DMBA), a potent carcinogen (Burchiel et al. 1988), developed skin cancer after 6 weeks, and reached a maximum tumour response after 12 weeks, while control animals exposed to the same compound developed no tumours (Chen et al. 2000). It has been extensively confirmed in the last decade that ODC overexpression is linked to cancerogenesis. A transgenic genetic model in which the expression of an inducible form of the ODC protein fused to a modified estrogen receptor ligand-binding domain (ODCER) that was driven by an involucrin promoter was engineered by Lan et al. (2005). In these transgenic mice, the treatment with the inducing agent 4-hydroxytamoxifen (4OHT) dramatically increased the ODC activity and Put levels in the epidermis and stimulated both proliferation in basal epidermal cells and differentiation (Lan et al. 2005). Therefore, although ODCER transgenic mice demonstrate a low basal ODC activity similar to wild-type mice and have a normal skin phenotype (Hayes et al. 2011), after 4OHT treatment and subsequent ODC induction, ODCER mice showed epidermal hyperproliferation with prolonged infiltration of inflammatory cells, activation of fibroblasts and increased vascularisation, growth factor and cytokine-enriched stroma that promoted tumourigenesis (Hayes et al. 2011). According to this study, it has been demonstrated that treatment of rodents with the α-difluoromethylornithine (DFMO), a specific inhibitor of ODC, reduces tumour progression in different animal models and the DFMO is currently being used in several ongoing chemoprevention trials (Gerner and Meyskens 2004). The evidence of a role for ODC in the tumour promotion of skin carcinogenesis was also provided from mice that constitutively express the AZ-1, a protein that reduces ODC and PA transport (Feith et al. 2001). These K6-AZ transgenic mice, overexpressing AZ-1, were noticeably more protected than wild-type controls in response to the treatment with the carcinogens DMBA and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a molecule demonstrated to be a potent tumour promoter in mouse skin (Fürstenberger et al. 1981). After double treatment with DMBA and TPA, the percentage of mice with tumours was ∼95 % for wild-type mice, but only 50–60 % for K6-AZ mice (Feith et al. 2007). Recently, Feith et al. (2013) have crossed K5-AZ-1 transgenic mice overexpressing AZ under the bovine keratin K5 promoter (Feith et al. 2001) with p53(±) mice to obtain double transgenic mice, which have been placed on a zinc-deficient or zinc-sufficient diet and chronically exposed to the tumourigenic compound 4-nitroquinoline1-oxide. Those mice showed an oral cavity and oesophagus cancer incidence lower than control animals, confirming the powerful tumour suppressive effects of targeted AZ expression and validate the PA biosynthetic pathway as a target for chemoprevention of tumour (Feith et al. 2013). Other studies suggest that also SSAT overexpression could be involved in tumourigenesis. K6-SSAT transgenic mice overexpressing SSAT, by means of the same genetic expression system (i.e. K6 promoter of bovine keratin VI), were engineered by Coleman et al. (2002). These K6-SSAT transgenic mice resulted more sensitive to tumour induction (Coleman et al. 2002). In agreement with previous studies carried out by Coleman and co-worker in 2002, Wang et al. (2007) confirmed that K6-SSAT mice are more prone in developing tumours after DMBA and TPA treatment, and that these tumours were significantly larger in transgenic animals than in wild-type controls. Since K6-AZ mice developed fewer tumours than syngenic mice, it was interesting to understand how the tumour suppressor role of AZ could affect the cancerogenesis in SSAT overexpressing mice. With this aim, after cross-breeding K6-SSAT with K6-AZ mice, the double transgenic animals K6-SSAT/K6-AZ mice, which have also an increase in AZ expression, showed a great reduction in the number and size of tumours (Wang et al. 2007). To test the effect of inhibiting ODC activity, K6-SSAT mice were also co-treated with DFMO, after DMBA and TPA administration, which caused a rapid regression of the tumours (Wang et al. 2007). Augmented levels of the intracellular concentrations of PAs have been also linked to tumour development in the gastrointestinal tract (Gerner and Meyskens 2004). The role of SSAT in intestinal tumourigenesis in vivo has been assayed crossing *Apc* $^{Min/+}$ mouse, a model of intestinal cancer (Moser et al. 1990), with genetically engineered mice either overexpressing (*Sat1*^{tg}) or lacking (*Sat1*$^{-}$) SSAT (Berger et al. 2007). In this genetic background (*Apc* $^{Min/+}$*Sat1*^{tg}), the increased PA

catabolism was associated with a compensatory increase in PA biosynthesis, since ODC and SAMDC were up-regulated. Interestingly, polyp numbers in both the small intestine and colon were increased in adult *Apc* $^{Min/+}$*Sat1*^{tg} relative to normal *Apc* $^{Min/+}$ mice, demonstrating that the SSAT overexpressing transgene promoted tumour development. The crossing between *Apc* $^{Min/+}$ mice with an SSAT-deficient strain (defective allele *Sat1*$^{-}$ of the endogenous *Sat1* gene) supported the involvement of SSAT in tumour progression. Double mutant mice exhibited a 75 % reduction in polyp numbers in the small intestine, as well as a compensatory reduction in ODC levels in adenomas, together with a modest decrease in Put content levels (Tucker et al. 2005). Surprisingly, SSAT overproduction was also associated to an inhibition of tumourigenesis. This link was investigated by the cross-breeding of SSAT overexpressing transgenic mice (Pietilä et al. 1997, 2001; Kee et al. 2004) with TRAMP mice, a model of prostate cancer (Greenberg et al. 1995). The progression of cancer in the TRAMP mouse was investigated by GU (genitourinary tracts consisting of bladder, urethra, seminal vesicles, ampullary gland, and the prostate) tracts weight (Kee et al. 2004). GU of the double transgenic TRAMP/SSAT mice was significantly smaller than those of the TRAMP controls. The first appearance of GU tumours occurs at 20 weeks of age in TRAMP animals and on the base of weight, TRAMP GU tracts were four times larger than those of TRAMP/SSAT mice. By 30 weeks, all TRAMP mice had visible prostate tumours and the disease index was found to be fivefold lower in TRAMP/SSAT mice than that of the TRAMP mice. Taken together, these results indicate that SSAT overexpression effectively suppresses tumour outgrowth in the TRAMP model (Kee et al. 2004). Changes in PA pathways in all these mouse models are dependent upon the nature of a particular cell's metabolome. The impact of SSAT expression on tumourigenesis can vary in different experimental models since it is context-dependent, and its effects on the other PA metabolic enzymes are expected to be highly environment related (Berger et al. 2007). This may explain why SSAT acts as a tumour promoter in the *Apc* $^{Min/+}$ model of intestinal cancer (Tucker et al. 2005) and as a tumour suppressor in the TRAMP model of prostate cancer (Kee et al. 2004).

Neurodegeneration

Additional studies with transgenic animals with modified PA metabolism have also contributed to resolve the persistent dilemma as to whether altered PA metabolism in response to CNS insults is a cause of neuronal damage or a sign of plasticity and neuroprotection (Jänne et al. 2005). Several studies have reported an increase of ODC activity and Put level in brain injury, such as ischaemia, excitotoxicity and trauma (Halonen et al. 1993). Although these results suggest that PAs play an important role in neurodegeneration, the mechanisms by which they participate in neuronal death are still unclear. The role of endogenous PAs in normal brain functioning is yet to be elucidated (Cervelli et al. 2012; Capone et al. 2013). In recent years, specific interactions of PAs with a number of different types of ion channels have been reported. These include the block of some types of K^+ channels and glutamate (Glu) receptors by intracellular PAs, and the modulation of other types of Glu receptors by extracellular PAs (Williams 1997; Traynelis et al. 2010). The role of PA in neurodegeneration has been investigated only by mice overexpressing ODC, SSAT and, very recently, SMO enzymes.

ODC overexpression

The first transgenic mouse line overexpressing ODC, assigned as K2, was generated in 1991, carrying a construct for the human-specific ODC mRNA expression in all tissues (Halmekytö et al. 1991). These mice displayed significant increases of ODC activity in a number of tissues: heart, lung, skeletal muscle and thymus. The most remarkable difference between syngenic and transgenic mice was, however, found in the brain; in fact brain of transgenic mice showed nearly 70 times higher ODC activity than that in syngenic mice (Halmekytö et al. 1991). In the K2 mouse line an exceedingly high Put concentration was found in the brain, while minimal changes were found in the levels of Spd and Spm. Examination of the transgenic animals with life-long overexpression of ODC and enhanced brain accumulation of Put did not reveal any signs of neuronal degeneration at the age of two years (Alhonen et al. 1995). In addition, these animals were also neuro-protected from physically or chemically induced seizure activity (Halonen et al. 1993). In fact, transgenic mice were protected from seizures induced by electroshock and by pentylenetetrazol (PTZ). However, those transgenic animals showed impaired spatial learning and memory as determined by water-maze test, characteristics involving employment of hippocampal structures and NMDA receptors (Halonen et al. 1993). Nevertheless, this phenotype is not caused by impaired vision, or swimming ability or lack of motivation, but rather represents a specific deficit in spatial learning associated with constitutively high Put and its antagonistic effect on NMDA receptor. In fact, it has been shown that both competitive and non-competitive antagonists of the NMDA-receptor impair, in a dose-dependent manner, the performance of rats in water-maze tests (Heale and Harley 1990; Kant et al. 1991). Moreover, when the ODC overexpressing rats were subjected to

transient focal cerebral ischaemia, the occlusion resulted in significantly smaller stroke lesions in transgenic rats in comparison with similarly treated non-transgenic rats (Lukkarinen et al. 1998). These results indicate that induction of ODC and the subsequent accumulation of Put are endogenous neuro-protective measures in transient cerebral ischaemia (Alhonen et al. 2009).

SSAT overexpression

The first transgenic mouse line ubiquitously overexpressing SSAT was generated by Pietilä et al. (1997). Similarly to ODC over-expressing animals, the SSAT mice displayed greatly expanded brain Put pools as a result of SSAT overexpression. These transgenic mice showed neuroprotection from KA-induced general and neuronal toxicity (Kaasinen et al. 2000) and an elevated threshold to PTZ-induced convulsions in comparison with wild-type animals (Kaasinen et al. 2003). Interestingly, the latter difference disappeared when the convulsant was administered concomitantly with ifenprodil, a *N*-methyl-D-aspartate (NMDA) antagonist (Kaasinen et al. 2003). Neurobehavioral profiling of SSAT overexpressing mice revealed that these animals were hypomotoric and less aggressive than wild-type animals and showed impaired spatial learning (Kaasinen et al. 2004). Spermidine and Spm interact as agonists at the NMDA receptor, while Put is believed to act as a weak antagonist (Williams et al. 1989, 1990). An elevated brain Put content could cause a partial blockade of the NMDA receptor, thus giving a rationale to explain the protection from seizure activity, ischaemia reperfusion damage and impaired spatial learning observed in transgenic animals.

SMO overexpression

A different scenario exhibits the very recently generated mouse genetic model overexpressing SMO, which provides novel evidences of the complex and critical functions carried out by SMO and Spm in mammalian brain (Cervelli et al. 2012). Spermine has been demonstrated to be the strongest PA modulator of GluRs, some types of K^+ channels and Na^+ channels (Williams 1997; Fleidervish et al. 2008; Traynelis et al. 2010). On this ground, a mouse line was engineered with the aim to investigate the effects of SMO overexpression in brain, up to now unexplored (Cervelli et al. 2013b). A Cre/*loxP*-based genetic model was constructed to obtain SMO overexpressing transgenic animals conditionally, i.e. when the mouse founder line (*JoSMO*), ubiquitously expressing GFP (Fig. 2), was bred with a transgenic line expressing the Cre recombinase in the brain neocortex. So, double transgenic mice (*JoSMOrec*) were obtained to specifically overexpress SMO in a

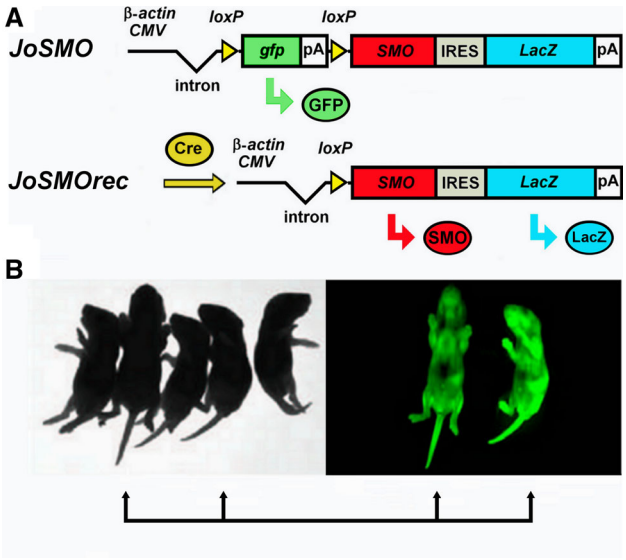

Fig. 2 Transgenic *JoSMO* and *JoSMOrec* mouse lines. **a** Scheme of *JoSMO* and, upon Cre recombination, of *JoSMOrec* genotypes. The β-actin/CMV fusion promoter drives the ubiquitous expression of the *gfp* (*green arrow*, Green Fluorescent Protein) reporter gene. Upon Cre recombination the *gfp*-stop cassette is excised, leading to simultaneous expression of *SMO* (*red arrow*) and of the second reporter gene, *lacZ* (*blue arrow*, β-galactosidase), via an IRES sequence. **b** *JoSMO* mice exhibited widespread GFP fluorescence (color figure online)

tissue-specific way, excluding any pleiotropic influence by other organs. Interestingly, *JoSMOrec* mice showed a phenotype with a significant astroglial and microglial activation in the neocortex of old animals, showing a more pronounced brain damage during ageing. In excitotoxic condition, KA injected *JoSMOrec* mice resulted to be more sensitive than control animals, showing more severe behavioural phenotype, astrogliosis and microgliosis in the neocortex and also a higher number of neurons with abnormal morphological features, all evident markers of brain injury (Fig. 3) (Cervelli et al. 2013b). Compared to transgenic ODC and SSAT overexpressing mice, which showed a neuroprotective response to different insults, *JoSMOrec* animals displayed an opposite phenotype, since an enhanced neurodegeneration during ageing and following KA injection has been observed (Alhonen et al. 2009; Cervelli et al. 2013b). In SMO overexpressing mice it has been observed a different Spm/Spd ratio that could affect GluRs, produces changes in Ca^{2+} flux through GluRs and be responsible for the higher sensitivity to KA treatment (Cervelli et al. 2013b). The production of H_2O_2 and AP, derived from Spm oxidation, together with direct effects of Spm on AMPA and KA receptors, are synergistically involved in ROS increase and ultimately to neuronal degeneration and death. To this regard, SMO enzyme can be considered as one of the most important H_2O_2 producers in the brain and the transgenic *JoSMOrec* mice represent a useful genetic model for studying brain

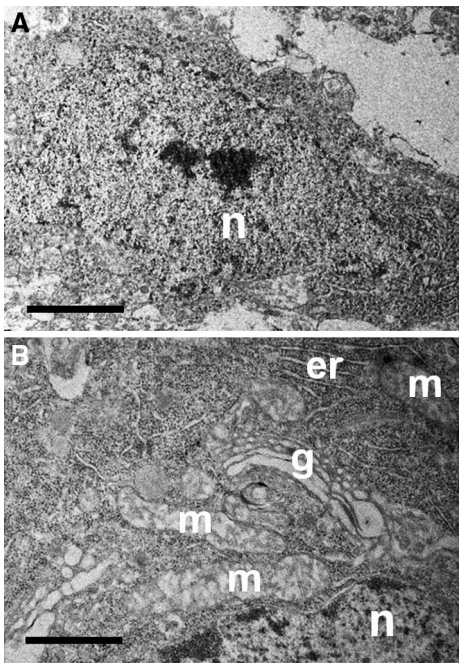

Fig. 3 Electron microscopic analysis of *JoSMOrec* neocortex**. a** Ultrathin section from neocortex of JoSMOrec mouse, showing cell morphology at ultrastructural level. Neuron shows abnormal morphological features, including cytoplasmic condensation and strong basophilia of the nucleus (*n*). **b** Cytoplasm of a damaged neuron showing enlargement of Golgi apparatus (*g*) and of endoplasmic reticulum cisternae (*er*), and swollen mitochondria (*m*). *Scale bars* 5 μm (**a**) and 2 μm (**b**)

pathologies such as epilepsy, Alzheimer's disease and other forms of dementia (Cervelli et al. 2013b).

Conclusions

Cloning of genes involved in PA metabolism has enabled the generation of genetically modified mice and rats overproducing or devoid of enzymes encoded by these genes. We reviewed the work carried out in inflammation, cancerogenesis and neurodegeneration and described the state of the art on transgenic animal models for PA research. Characterisation of these transgenic animal phenotypes has revealed a multitude of physiological changes, many of which could not have been foreseen from the previous knowledge of the PA requirements and functions (Table 1).

As shown by the articles reviewed, inflammatory processes are affected in a complex way by alteration of PA metabolism. Polyamines appear to possess a protective role in tissues, since in general their depletion lead to inflammation (Hyvönen et al. 2007). The inflammation induced by SSAT overexpression in transgenic mice appears to be mediated by the induction of trypsinogen activation driven by a Spd and Spm content decrease. The pro-inflammatory role of SSAT enzyme in response to LPS is confirmed by SSAT-deficient mice, which appear to be more protected in comparison to wild-type controls (Zahedi et al. 2010). On the other hand, a further research on transgenic mice overexpressing SSAT treated with LPS has shown an anti-inflammatory effect, since the level of serum pro-inflammatory cytokines was observed to decrease (Pirnes-Karhu et al. 2012). This contradiction indicates the complexity of the inflammatory response in relation to the SSAT expression.

Altered PA metabolism in tumourigenesis has been investigated mainly with ODC overexpressing and SSAT overexpressing/knockout genetic models. The increase in ODC activity in inducible and constitutively overexpressing transgenic mouse models seems to increase (Chen et al. 2000) or promote tumourigenesis after tumour induction by chemical substances (Hayes et al. 2011). In agreement with these results, transgenic mice which overexpress AZ-1 showed a decrease susceptibility to carcinogens (Feith et al. 2007), as well as the use of the specific ODC inhibitor leaded to tumour reduction in animal models (Gerner and Meyskens 2004). However, the use of overexpressing/knockout transgenic animals in carcinogenesis experiments shows a context-dependent SSAT role, since its expression can reduce or promote tumour development according to the effect induced on PA metabolism (Tucker et al. 2005; Gilmour 2007; Wang et al. 2007; Alhonen et al. 2009; Feith et al. 2013).

Studies on transgenic animals with modified PA metabolism have also contributed to elucidating the involvement of PAs in brain functioning and pathology. Transgenic animals overexpressing ODC and SSAT showed an enhanced brain Put accumulation which has been demonstrated to have a neuroprotective role. In fact using these transgenic animals it has been observed that Put increase elevates seizure threshold (Halmekytö et al. 1993; Halonen et al. 1993; Kaasinen et al. 2003) and protects against ischaemia–reperfusion damage (Lukkarinen et al. 1998; Jänne et al. 2005), but leads to impair spatial learning (Halonen et al. 1993; Kaasinen et al. 2004). Since functional NMDA receptors are needed for synaptic plasticity and spatial learning (Paschen 1992), and their prolonged activation is associated with neuronal damage, expanded brain Put accumulation in the transgenic animals is protective being responsible of partial blockade of NMDA receptors (Jänne et al. 2005). Recently, to investigate the neurobiological role of Spm, a transgenic mouse line was engineered to specifically overexpress SMO in the brain neocortex (Cervelli et al. 2013b). The effect of SMO overexpression in the neocortex leads to a phenotype which showed a significant astroglial and microglial activation in old animals, together with a more pronounced brain

damage during ageing. Moreover, *JoSMOrec* mice display a higher sensitiveness to KA injection than control mice, as demonstrated by the behavioural phenotype analysis, immunodistribution of neural and glial cell populations, and an increase of abnormal neurons. The *JoSMOrec* neocortex phenotype observed could be due to a decrease of the Spm/Spd ratio which affects glutamatergic transmission and/or to a higher H_2O_2 and acrolein production, both responsible for a severe cellular stress. The neuronal damage derived from acrolein, which is considered as a marker of brain injuries, is corroborated by many evidences including experiments carried out on a strain of mouse called Gyro mice (Gy), where a deletion on the X chromosome has eliminated the *SMS* gene (Lyon et al. 1986). Gyro mice have been utilised in stroke experiments, indicating that the toxic effects of acrolein were effectively derived from SMO and not from SSAT and APAO activities (Saiki et al. 2011). All together, these evidences indicate an important role of SMO during excitotoxicity, ischaemia and neuronal damages and provide new perspectives on the complex and critical functions carried out by SMO and Spm in the physiology and pathology of mammalian brain. Furthermore, designing of PA analogues able to inhibit SMO activity and/or modulate Glu receptors will be of great relevance to select new potential drugs with brain neuroprotective activity.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Alhonen L, Halmekytö M, Kosma VM, Wahlfors J, Kauppinen R et al (1995) Life-long over-expression of ornithine decarboxylase (ODC) gene in transgenic mice does not lead to generally enhanced tumorigenesis or neuronal degeneration. Int J Cancer 63:402–404

Alhonen L, Parkkinen JJ, Keinänen T, Sinervirta R, Herzig KH et al (2000) Activation of polyamine catabolism in transgenic rats induces acute pancreatitis. Proc Natl Acad Sci USA 97:8290–8295

Alhonen L, Uimari A, Pietilä M, Hyvönen MT, Pirinen E et al (2009) Transgenic animals modelling polyamine metabolism-related diseases. Essays Biochem 46:125–144

Amendola R, Cervelli M, Fratini E, Polticelli F, Sallustio DE et al (2009) Spermine metabolism and anticancer therapy. Curr Cancer Drug Targ 9:118–130

Bellelli A, Cavallo S, Nicolini L, Cervelli M, Bianchi M et al (2004) Mouse spermine oxidase: a model of the catalytic cycle and its inhibition by *N, N1*-bis(2,3-butadienyl)-1,4-butanediamine. Biochem Biophys Res Commun 322:1–8

Berger FG, Kramer DL, Porter CW (2007) Polyamine metabolism and tumorigenesis in the Apc(Min/+) mouse. Biochem Soc Trans 35:336–339

Bianchi M, Polticelli F, Ascenzi P, Botta M, Federico R et al (2006) Inhibition of polyamine and spermine oxidases by polyamine analogues. FEBS J 273:1115–1123

Burchiel SW, Hadley WM, Barton SL, Fincher RH, Lauer LD et al (1988) Persistent suppression of humoral immunity produced by 7,12-dimethylbenz(a)anthracene (DMBA) in B6C3F1 mice: Correlation with changes in spleen cell surface markers detected by flow cytometry. Int J Immunopharmacol 10:369–376

Capone C, Cervelli M, Angelucci E, Colasanti M, Macone A, Mariottini P, Persichini T (2013) A role for spermine oxidase as a mediator of reactive oxygen species production in HIV-Tat-induced neuronal toxicity. Free Radic Biol Med 63C:99–107

Cervelli M, Polticelli F, Federico R, Mariottini P (2003) Heterologous expression and characterization of mouse spermine oxidase. J Biol Chem 278:5271–5276

Cervelli M, Amendola R, Polticelli F, Mariottini P (2012) Spermine oxidase: ten years after. Amino Acids 42:441–450

Cervelli M, Salvi D, Polticelli F, Amendola R, Mariottini P (2013a) Structure-function relationships in the evolutionary framework of spermine oxidase. J Mol Evol. doi:10.1007/s00239-013-9570-3. (in press)

Cervelli M, Bellavia G, D'Amelio M, Cavallucci V, Moreno S et al (2013b) A new transgenic mouse model for studying the neurotoxicity of spermine oxidase dosage in the response to excitotoxic injury. PLoS One 8:e64810

Chen Y, Megosh LC, Gilmour SK, Sawicki JA, O'Brien TG (2000) K6/ODC transgenic mice as a sensitive model for carcinogen identification. Toxicol Lett 116:27–35

Coleman CS, Pegg AE, Megosh LC, Guo Y, Sawicki JA et al (2002) Targeted expression of spermidine/spermine N1-acetyltransferase increases susceptibility to chemically induced skin carcinogenesis. Carcinogenesis 23:359–364

Feith DJ, Shantz LM, Pegg AE (2001) Targeted antizyme expression in the skin of transgenic mice reduces tumor promoter induction of ornithine decarboxylase and decreases sensitivity to chemical carcinogenesis. Cancer Res 61:6073–6081

Feith DJ, Shantz LM, Shoop PL, Keefer KA, Prakashagowda C et al (2007) Mouse skin chemical carcinogenesis is inhibited by antizyme in promotion-sensitive and promotion-resistant genetic backgrounds. Mol Carcinog 46:453–465

Feith DJ, Pegg AE, Fong LY (2013) Targeted expression of ornithine decarboxylase antizyme prevents upper aerodigestive tract carcinogenesis in p53-deficient mice. Carcinogenesis 34:570–576

Fleidervish IA, Libman L, Katz E, Gutnick MJ (2008) Endogenous polyamines regulate cortical neuronal excitability by blocking voltage-gated Na+ channels. Proc Natl Acad Sci USA 105:18994–18999

Fürstenberger G, Berry DL, Sorg B, Marks F (1981) Skin tumor promotion by phorbol esters is a two-stage process. Proc Natl Acad Sci 78:7722–7726

Gerner EW, Meyskens FL Jr (2004) Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 4:781–792

Gilmour SK (2007) Polyamines and nonmelanoma skin cancer. Toxicol Appl Pharmacol 224:249–256

Gobert AP, Cheng Y, Wang JY, Boucher JL, Iyer RK et al (2002) *Helicobacter pylori* induces macrophage apoptosis by activation of arginase II. J Immunol 168:4692–4700

Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD et al (1995) Prostate cancer in a transgenic mouse. Proc Natl Acad Sci USA 92:3439–3443

Halmekytö M, Hyttinen JM, Sinervirta R, Utriainen M, Myöhänen S et al (1991) Transgenic mice aberrantly expressing human ornithine decarboxylase gene. J Biol Chem 266:19746–19751

Halmekytö M, Alhonen L, Alakuijala L, Jänne J (1993) Transgenic mice over-producing putrescine in their tissues do not convert the diamine into higher polyamines. Biochem J 291:505–508

Halonen T, Sivenius J, Miettinen R, Halmekytö M, Kauppinen R et al (1993) Elevated seizure threshold and impaired spatial learning in transgenic mice with putrescine overproduction in the brain. Eur J Neurosci 5:1233–1239

Hayes CS, Defeo K, Dang H, Trempus CS, Morris RJ et al (2011) A prolonged and exaggerated wound response with elevated ODC activity mimics early tumor development. Carcinogenesis 32:1340–1348

Heale V, Harley C (1990) MK-801 and AP5 impair acquisition, but not retention, of the Morris milk maze. Pharmacol Biochem Behav 36:145–149

Hyvönen MT, Merentie M, Uimari A, Keinänen TA, Jänne J et al (2007) Mechanisms of polyamine catabolism-induced acute pancreatitis. Biochem Soc Trans 35:326–330

Jänne J, Alhonen L, Keinänen TA, Pietilä M, Uimari A et al (2005) Animal disease models generated by genetic engineering of polyamine metabolism. J Cell Mol Med 9:865–882

Kaasinen SK, Gröhn OH, Keinänen TA, Alhonen L, Jänne J (2000) Over-expression of spermidine/spermine *N*1-acetyltransferase elevates the threshold to pentylenetetrazol-induced seizure activity in transgenic mice. Exp Neurol 183:645–652

Kaasinen SK, Gröhn OH, Keinänen TA, Alhonen L, Jänne J (2003) Overexpression of spermidine/spermine *N*1-acetyltransferase elevates the threshold to pentylenetetrazol-induced seizure activity in transgenic mice. Exp Neurol 183:645–652

Kaasinen SK, Oksman M, Alhonen L, Tanila H, Jänne J (2004) Spermidine/spermine *N1*-acetyltransferase overexpression in mice induces hypoactivity and spatial learning impairment. Pharmacol Biochem Behav 78:35–45

Kant GJ, Wright WL, Robinson TN 3rd, D'Angelo CP (1991) Effects of MK-801 on learning and memory as assessed using a novel water maze. Pharmacol Biochem Behav 39:479–485

Kee K, Foster BA, Merali S, Kramer DL, Hensen ML et al (2004) Activated polyamine catabolism depletes acetyl-CoA pools and suppresses prostate tumor growth in TRAMP mice. J Biol Chem 279:40076–40083

Lan L, Hayes CS, Laury-Kleintop L, Gilmour SK (2005) Suprabasal induction of ornithine decarboxylase in adult mouse skin is sufficient to activate keratinocytes. J Invest Dermatol 124: 602–614

Lukkarinen JA, Kauppinen RA, Gröhn OH, Oja JM, Sinervirta R et al (1998) Neuroprotective role of ornithine decarboxylase activation in transient cerebral focal ischemia: A study using ornithine decarboxylase-overexpressing transgenic rats. Eur J Neurosci 10:2046–2055

Lyon MF, Scriver CR, Baker LR, Tenenhouse HS, Kronick J et al (1986) The Gy mutation: another cause of X-linked hypophosphatemia in mouse. Proc Natl Acad Sci USA 83:4899–4903

Megosh L, Gilmour SK, Rosson D, Soler AP, Blessing M et al (1995) Increased frequency of spontaneous skin tumors in transgenic mice which overexpress ornithine decarboxylase. Cancer Res 55:4205–4209

Moser AR, Pitot HC, Dove WF (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247:322–324

Paschen W (1992) Polyamine metabolism in different pathological states of the brain. Mol Chem Neuropathol 16:241–271

Pegg AE, Feith DJ, Fong LY, Coleman CS, O'Brien TG et al (2003) Transgenic mouse models for studies of the role of polyamines in normal, hypertrophic and neoplastic growth. Biochem Soc Trans 31:356–360

Pietilä M, Alhonen L, Halmekytö M, Kanter P, Jänne J et al (1997) Activation of polyamine catabolism profoundly alters tissue polyamine pools and affects hair growth and female fertility in transgenic mice overexpressing spermidine/spermine *N1*-acetyltransferase. J Biol Chem 272:18746–18751

Pietilä M, Parkkinen JJ, Alhonen L, Jänne J (2001) Relation of skin polyamines to the hairless phenotype in transgenic mice overexpressing spermidine/spermine *N*-acetyltransferase. J Invest Dermatol 116:801–805

Pirnes-Karhu S, Sironen R, Alhonen L, Uimari A (2012) Lipopolysaccharide-induced anti-inflammatory acute phase response is enhanced in spermidine/spermine *N1*-acetyltransferase (SSAT) overexpressing mice. Amino Acids 42:473–484

Polticelli F, Salvi D, Mariottini P, Amendola R, Cervelli M (2012) Molecular evolution of the polyamine oxidase gene family in Metazoa. BMC Evol Biol 12:90

Rasanen TL, Alhonen L, Sinervirta R, Keinänen T, Herzig KH et al (2002) A polyamine analogue prevents acute pancreatitis and restores early liver regeneration in transgenic rats with activated polyamine catabolism. J Biol Chem 277:39867–39872

Saiki R, Park H, Ishii I, Yoshida M, Nishimura K et al (2011) Brain infarction correlates more closely with acrolein than with reactive oxygen species. Biochem Biophys Res Commun 404:1044–1049

Tavladoraki P, Cervelli M, Antonangeli F, Minervini G, Stano P et al (2011) Probing mammalian spermine oxidase enzyme-substrate complex through molecular modeling, site-directed mutagenesis and biochemical characterization. Amino Acids 40:1115–1126

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM et al (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62:405–496

Tucker JM, Murphy JT, Kisiel N, Diegelman P, Barbour KW et al (2005) Potent modulation of intestinal tumorigenesis in Apcmin/+ mice by the polyamine catabolic enzyme spermidine/spermine N1-acetyltransferase. Cancer Res 65:5390–5398

Uimari A, Merentie M, Sironen R, Pirnes-Karhu S, Peräniemi S et al (2012) Overexpression of spermidine/spermine *N1*-acetyltransferase or treatment with N1-N11-diethylnorspermine attenuates the severity of zinc-induced pancreatitis in mouse. Amino Acids 42:461–471

Wang X, Feith DJ, Welsh P, Coleman CS, Lopez C et al (2007) Studies of the mechanism by which increased spermidine/spermine *N1*-acetyltransferase activity increases susceptibility to skin carcinogenesis. Carcinogenesis 28:2404–2411

Williams K (1997) Interactions of polyamines with ion channels. Biochem J 325:289–297

Williams K, Dawson VL, Romano C, Dichter MA, Molinoff PB (1990) Characterization of polyamines having agonist, antagonist, and inverse agonist effects at the polyamine recognition site of the NMDA receptor. Neuron 5:199–208

Williams K, Romano C, Molinoff PB (1989) Effects of polyamines on the binding of [3H]MK-801 to the *N*-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. Mol Pharmacol 36:575–581

Zahedi K, Barone S, Kramer DL, Amlal H, Alhonen L et al (2010) The role of spermidine/spermine *N1*-acetyltransferase in endotoxin-induced acute kidney injury. Am J Physiol Cell Physiol 299:164–174

Zhang M, Wang H, Tracey KJ (2000) Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. Crit Care Med 28:60–66